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Microbiology of Charcoal-Broiled European River Lampreys (*Lampetra fluviatilis*) Stored at 3 and 22°C

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ABSTRACT

The microbiological quality of 30 production lots of charcoal-broiled river lampreys was studied at three lamprey processing plants (plants A, B, and C). Samples were taken directly after charcoal broiling and stored at 22 and 3°C. Lampreys were examined on the day of manufacture, and those kept at 22°C were examined every second day for 6 days. Samples kept at 3°C were examined every fourth day for up to 24 days. On the production day, the mean aerobic plate counts (APCs) for broiled lampreys from plants A, B, and C were 2.29 log CFU/g, 1.88 log CFU/g, and undetectable (1.67 log CFU/g), respectively. At 22°C, the mean APCs for samples from plants A, B, and C increased markedly within 4 days, and after 6 days the counts for samples from these plants were 8.56, 5.04, and 6.23 log CFU/g, respectively. Chilling and storage at 3°C remarkably improved the shelf life of the product. The levels of bacteria in charcoal-broiled river lampreys from plant A were higher than those in lampreys from plants B and C. No significant increases in APCs were observed during storage at 3°C for 24 days; mean APCs did not exceed 2.80 log CFU/g for samples from any plant. *Staphylococcus aureus* was found in two samples. No lactic acid bacteria, thermotolerant coliforms, enterococci, *Clostridium perfringens,* or *Listeria monocytogenes* was detected. Microbiological data from this study will be used for the development of a hazard analysis for the determination of critical control points.

The charcoal-broiled river lamprey is a specialty food in the Baltic Sea region. Because of the traditional processing method for lampreys, this product differs remarkably from similar fish products. The European river lamprey (*Lampetra fluviatilis*) is common along the coast of Finland, and 28 rivers are known to support spawning populations. The river lampreys feed on valuable fish species such as Baltic herring, sprat, and vendace *(3).* The annual lamprey catch in Finnish coastal rivers comprises about 1,500,000 to 2,000,000 fish. Lampreys are used only for human consumption *(3, 13).* Lamprey manufacturers are very small family enterprises with staffs of only two or three persons. Lampreys are consumed as a delicacy directly from lamprey booths or in restaurants as components of hors d'oeuvres.

The spawning migration of lampreys from the sea to the rivers begins in August. September and October are the main months for migration, which continues to decrease until February *(13).* In the beginning of their migration, lampreys stop feeding *(3).* The energy for migration, development of the gonads, and spawning is generated from fat deposits, which may account for up to 44 and 47% of the dry weights of females and males, respectively *(5).* Since there is no food in the lampreys' guts, their intestinal flora is greatly reduced. The fishing season begins in August and ends in December. After spawning, the energy deposits of the lampreys are exhausted and they die *(5).*

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The microbial flora associated with lampreys and lamprey products is practically unknown. The aim of this study was to characterize the major bacterial groups detected in charcoal-broiled river lampreys stored at 22 and 3°C. A temperature of 22°C was chosen because the product is traditionally brought to the market without chilling. We also set out to compare differences between three manufacturers with respect to the development of microbial groups.

MATERIALS AND METHODS

Processing of lampreys. The charcoal broiling procedures used at the three plants studied were traditional, originating from earlier centuries. The methods and the expertise used to produce charred lamprey are handed down from generation to generation. Lampreys are rubbed in the round with salt in an apparatus resembling a concrete mixer. The apparatus is made from stainless steel and motorized. The process is carried out at room temperature.

Manufacturer A adds 1,300 g (1 liter) of crystalline NaCl for every 40 kg of lamprey, and manufacturer B uses about 1 kg of salt for every 50 kg of lamprey, although the amount of salt used depends on the consistency and the origin of the lampreys. The amount of salt used in plant C is 1 kg for every 50 kg of lamprey. The mixing time for the running apparatus ranges from 1 to 1.5 h depending on the size, color, and origin of the lampreys. After salting, the lampreys are rinsed under running tap water (controlled by municipal authorities) for 5 min. After a waiting period of 12 h, the lampreys are broiled in ovens on charcoal made from alder. The broiling time varies depending on the master in charge, and the total time varies from 20 to 30 min. Chilling occurs at room temperature, and the product is then delivered to the market without any more processing.

TABLE 1. *Aerobic bacterial counts for charcoal-broiled river lamprey samples stored at 22 and 3°C*

Temp (°C)	Storage time (days)	APC (log CFU/g) for samples from plant[a]: A[b]	B	C
22	0	2.29 (ND–5.68)	1.88 (ND–3.50)	ND
	2	2.82 (ND–5.65)	2.29 (ND–5.25)	2.82 (ND–5.14)
	4	7.45 (5.50–8.90)	3.26 (ND–7.54)	4.99 (ND–9.07)
	6	8.56 (ND–9.57)	5.04 (ND–9.36)	6.23 (ND–9.08)
3	4	2.82 (ND–8.47)	1.96 (ND–4.39)	ND
	8	2.36 (ND–8.27)	2.62 (ND–6.69)	ND
	12	2.35 (ND–8.17)	1.78 (ND–2.60)	ND
	16	2.36 (ND–8.31)	1.87 (ND–3.38)	ND
	20	2.80 (ND–8.31)	ND	ND
	24	2.36 (ND–8.31)	ND	ND

[a] Mean value for 10 different production runs, with range in parentheses. ND, not detected (assigned a value of 0.5× detection limit [2.0 log CFU/g] for calculation of mean).

[b] The APC at 3°C for one of the production lots exceeded 8.0 log CFU/g after 4 days, and the value used after 12 days was 8.31 log CFU/g.

Sample collection. Ten production lots from each manufacturer were examined during the autumn of 1998. Each lot consisted of 20 lampreys and was divided into two parts and put into plastic bags that were permeable to air under aseptic conditions. The time from collection of the lampreys to the start of the storage study did not exceed 2 h. Half of the samples were kept at 22°C, and the other half were kept at 3°C. The samples stored at 22°C were examined on days 0, 2, 4, and 6 or until the aerobic bacterial counts stopped rising, and the samples stored at 3°C were examined on days 4, 8, 12, 16, 20, and 24. A sample consisted of one whole lamprey. A total of 300 lampreys were studied.

Microbiological methods. A 10-g transversal-slice sample of a lamprey was placed in a sterile stomacher bag (Seward Ltd., London, UK) with 90 ml of saline-peptone solution (0.85% NaCl, 0.1% peptone; Maximal Recovery Diluent, Lab M Ltd., Bury, UK) and homogenized with a stomacher (Lab-Blender 400, Seward). The homogenized sample dilution was serially diluted with 0.9 ml of saline-peptone solution (Lab M) dilution blanks. Each dilution was plated onto the appropriate media by the pour or spread plate technique.

The aerobic plate count (APC) was determined by the ISO method *(4)* with the use of plate count agar (Oxoid, Hampshire, UK). Lactic acid bacteria, enterococci, and thermotolerant coliforms were determined by the methods of the Nordic Committee on Food Analysis (NCFA) *(8, 9, 11)* with the use of deMan Rogosa Sharpe agar (Oxoid), Slanetz and Bartley agar (Oxoid), and violet red bile agar (Oxoid), respectively. *Staphylococcus aureus* counts were determined with the use of Baird-Parker agar (Oxoid) *(10)*, and the suspected colonies were confirmed with apiSTAPH (bioMérieux, Marcy d'Etoile, France). *S. aureus* SLV-350 was used as a positive control. *Clostridium perfringens* counts were determined with perfringens agar (Oxoid) *(12)*. *C. perfringens* EELA 3/96 was used as a positive control. The isolation of *Listeria monocytogenes* was carried out according to the NCFA method *(7)* with Oxford Listeria selective agar plates (Oxoid). *L. monocytogenes* EELA was used as a positive control.

Statistical analysis. Student's t test and the χ^2 test were used. When the growth level was below the detection limit (2.0 log CFU/g), the value used in statistical calculations was 1.7 log CFU/g.

RESULTS

The mean APC on the day of production for charcoal-broiled river lamprey samples from plant A was 2.29 log CFU/g, and that for samples from plant B was 1.88 log CFU/g. For samples from plant C, the growth level for every production lot was below the detection limit (2.0 log CFU/g). The development of APCs at different temperatures is shown in Table 1.

After the first 2 days of storage at 22°C, the mean APCs for the products from plants A, B, and C did not exceed 2.82, 2.29, and 2.82 log CFU/g, respectively. For samples stored at 22°C, the mean APCs for samples from plant A were significantly higher than those for samples from plants B and C ($P < 0.05$) on days 4 and 6. There were no statistically significant differences in between mean log bacterial counts for plants B and C. The percentages of samples with APCs of >7.0 log CFU/g after 4 days of storage were 60, 10, and 30% for samples from plants A, B, and C, respectively. After 6 days of storage, these percentages were 60, 40, and 40%, respectively. There were significant differences between all producers after 4 days of storage, and after 6 days, significant differences between plants A and B and between plants A and C ($P < 0.05$) were observed. When we calculated the percentages of samples for which APCs exceeded the detection limit (2.0 log CFU/g), we found that the percentage for samples from plant A (65%) was statistically higher than those for samples from plants B (35%) and C (43%) ($P < 0.05$).

For lamprey samples stored at 3°C, the highest mean APCs were those for samples from plants A and B (2.82 and 2.62 log CFU/g, respectively). For all samples from plant C, the APCs were below the detection limit. The mean APC for one production lot from plant A exceeded 8.0 log CFU/g after 4 days of storage. There were no statistical differences between APCs for the different plants. We found that the percentages of samples with APCs exceeding the detection limit were 15, 8, and 0% for plants A, B, and C, respectively. The percentage for plant C was statistically

significantly lower than those for plants A and B ($P < 0.05$). *S. aureus* was isolated from a sample from plant A that had been stored for 4 days at 3°C (3.57 log CFU/g) and from a sample from plant B that had been stored for 2 days at 22°C (4.95 log CFU/g). The numbers of lactic acid bacteria, thermotolerant coliforms, and enterococci were below the detection limits (2.0, 1.0, and 1.0 log CFU/g, respectively). No *C. perfringens* or *L. monocytogenes* was detected in any of the samples studied.

DISCUSSION

The microbiological quality of charcoal-broiled river lampreys varied depending on the plant where they were processed. Lamprey processing methods are not standardized, even within any one plant. The quality of the raw lamprey apparently has an influence on the process. The charring time and temperature are controlled only by the food processor. The master in charge evaluates the size and color of the lamprey, and even the river the lamprey was caught from is taken into account before the charring procedure is chosen. In plant A, the common practice is to preserve the moistness of the product to achieve a "juicier" taste. The food processors in plant B and especially in plant C prefer a drier, crispier product. These preferences may have an effect on the different rates of the deterioration of the products. For samples stored at 22°C, the microbial level for samples from plant A was higher than those for samples from plants B and C. Plant A's high microbial level may be a result of an insufficient broiling procedure. For samples from plant C stored at 3°C, aerobic bacterial counts were below the detection limit regardless of storage time.

It is important to determine whether a reduced level of microorganisms means a low rate of microbial development in broiled lampreys during the first 2 days of storage even at 22°C. Such a determination is crucial because the product is brought to the market without chilling. We found that the APC for charred river lamprey samples did not exceed 7.0 log CFU/g during the first 2 days even when the samples were stored at 22°C. After 4 days of storage, APCs exceeded 7.0 log CFU/g for 60% of the samples. This finding indicates that the traditional nonchilling practice seems to result in the deterioration of the product after 2 days.

No growth of thermotolerant coliform bacteria or fecal streptococci was detected, perhaps because of the heavy heat treatment (20 to 30 min on red-hot charcoal) used for the product. The river lampreys stop feeding at the beginning of their spawning migration in August and September, which is the period during which they swim from the sea into the rivers, where they are caught *(13)*. Therefore, the intestinal contents are low and the microbial populations are small in lampreys caught during migration. This explains the relatively low level of microbial development of the product even when it is stored at 22°C and is probably why microbiological changes in lampreys differ from those seen in similar products such as charred Baltic herring. Charred Baltic herring have been found to contain excessive amounts of coliform bacteria and fecal streptococci after 48 and 96 h of storage at 20°C *(6)*.

In the present study, *S. aureus* was the only pathogenic bacterium isolated. This bacterium was found in two samples (0.6% of the total). It was probably introduced into the product by workers' hands or utensils. Hatakka et al. *(1)* found out that there was a prevalence of *S. aureus* in nasal samples (29%) and hand samples (9%) obtained from flight-catering employees. Our finding, however, suggests that the product may present a risk when it is not chilled and stored at temperatures lower than the traditional 22°C. Because the product is not systematically chilled, it is possible that *S. aureus* may grow during the transport and retail stages and cause food poisoning. Smoked and charred Baltic herring have been reported to be a common cause of staphylococcal food poisoning outbreaks *(2)*. Also, the storage of lampreys at 3°C was found to increase the shelf life of the product by up to 24 days except for one lot from plant A that apparently was not processed properly. During this sampling period, a part-time worker was employed at plant A. This finding might constitute evidence that charcoal broiling demands special professional skills.

A central tenet of food preservation is control of the temperature of the food product. The present study clearly demonstrates the advantage that would be obtained with storage at 3°C (the temperature commonly recommended for fish and fishery products) compared with the traditionally used 22°C. Chilled storage would result in crucial changes in the shelf life of the product. Apparently, the common habit of consuming lampreys on the day of production does not pose a health risk. It seems that large numbers of bacteria isolated from river lampreys arise from factors associated with lamprey processing, such as processing time and workers. Microbiological data from this study will be used for the development of hazard analysis for the implementation of the hazard analysis critical control point program for processed river lampreys. The present study also further underscores the need to implement the current regulations for the supervision of traditionally produced fishery products.

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